Time and Inhibitory Learning

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April 2011

Abstract

Associative theories of learning state that an organism acquires new behaviors when associations are formed between a Conditioned Stimulus (CS) and an Unconditioned Stimulus (US). Alternative theories suggest that learning is not reliant on associations between stimuli, but rather on the temporal information conveyed by stimuli. Studies have shown that temporal information influences Excitatory Learning. However, little is known about how time affects Inhibitory Learning, or the learning that occurs during periods of nonreinforcement. In two experiments, rats were exposed to auditory tones of varying durations, receiving appetitive reinforcers solely in the absence of the tones. The first experiment examined the effect of fixed CS- durations on inhibitory responding, while the second examined the effect of variable CS- durations on inhibitory responding. Results show that responding was dependent on the duration of the auditory CS-, as well as whether or not the CS- was of a fixed or variable duration, indicating that time influences inhibitory learning.

Introduction

The study of adaptive behavior, which is believed to occur when an organism learns about information conveyed by environmental events, can reveal insight into the ways by which organisms alter their behaviors to changing environments. For instance, an organism may learn that certain stimuli signal the presence or the absence of other naturally rewarding or naturally punitive stimuli. This learned information is subsequently used to guide behaviors, allowing the organism to exhibit responses, or to inhibit responses, during intervals of time for which stimuli signal that it is beneficial to do so. Consider the following applied example involving an organism who learns that a neutral environmental stimulus signals either the availability or the

unavailability of nutritional resources. When the stimulus signals food availability, the organism will engage in food-seeking behavior during the stimulus's presence and inhibit food-seeking behavior during its absence. Conversely, when the stimulus signals food unavailability, the organism will engage in food-seeking behavior during its absence and will inhibit behavior during its presence. By learning about the information that is signaled by cues, an organism acquires the important skill of discriminating between cues that indicate the presence and the absence of rewards and punishments, ultimately allowing it to adopt behaviors that are appropriate and useful to specific environmental conditions.

The learning of adaptive behaviors is formally characterized in terms of excitatory and inhibitory conditioning. Excitatory conditioning consists of repeated pairings of a Neutral Stimulus (NS) and an Unconditioned Stimulus (US). The US consists of a stimulus that is either intrinsically pleasurable or harmful; thus, standing on its own the US is capable of evoking responses, known as Unconditioned Responses (UR). After repeated pairings of the NS and US, the NS transforms into a Conditioned Stimulus (CS), which, on its own, can evoke behaviors characterized by the UR. However, when evoked by the CS, the behavior is said to be a Conditioned Response (CR). Inhibitory conditioning occurs when a NS is repeatedly paired with the absence of a US, resulting in the transformation of the NS into a CS that elicits a CR of inhibiting the behavior normally associated with the US (Pavlov, 1927).

The mechanisms underlying the processes of associative learning have been studied extensively since the early 20th century. Currently, the leading theory accounting for the acquisition of conditioned behavior, the Rescola-Wagner Model of Learning, is based on principles of involving the contingency of the relationships between environmental events. The term "contingency" refers to the probability that an animal will receive a US during a CS,

compared to the probability that it will receive a US in a CS's absence. According to Rescorla, a higher probability of US occurrence during periods of the CS, relative to periods of the CS's absence, will result in excitatory conditioning. Conversely, a higher probability of US occurrence during periods devoid of the CS, relative to periods in the CS's presence, will produce inhibitory conditioning (Rescorla, 1968a; Rescorla, 1968b).

In 1968(b), Rescorla tested this theory by conducting an experiment that examined the effects of contiguous CS-US relationships and of the numerical quantity of CS-US pairings on the strength of conditioned behavior. Using a fear conditioning paradigm with rats, the first experiment consisted of one group of animals that received shocks only during the CS period, therefore experiencing a positive contingency. The remaining two groups received shocks at equal rates in the presence and absence of CS, therefore experiencing zero contingency; however, the groups differed in that one received twice as many overall CS-US pairings than the other. Results showed a successful exhibition of conditioned fear responses by rats in the group that received shocks in a positively contingent manner, and a failure to display conditioned fear responses in the two control groups, leading to the conclusion that CS-US contingency, rather than the number of CS-US pairings, is responsible for conditioned behavior. Subsequent studies by Rescorla examined a wide array of intermediary levels of contingency and found that greater positive discrepancies between the probability of the US in the presence of the CS and the probability of the US in the CS's absence produce stronger excitatory conditioning (1968b).

Similar results were obtained by Rescorla in studies that examined negative CS-US contingencies to form related conclusions regarding the important role of contingency in associative learning (1968a). Using the fear-conditioning paradigm, rats were exposed to shocks at varying rates in the absence of the CS, thereby creating negative contingencies, as the rate of

US presentation during the absence of the CS was higher than during the presence of the CS. Rats that received the highest rate of shock in the absence of the CS displayed the strongest inhibition of fearful behavior in response to the onset of the CS, while rats that received the lowest rate of shock during the absence of the CS displayed the weakest inhibition of fear; rats that received shock during the absence of the CS at rates in between the highest and lowest rates possible, exhibited inhibitory behavior at levels in between those of the two extremes (1968a).

The effects of contingency in an excitatory and inhibitory setting are explained mechanistically in the Rescorla-Wagner Model of Learning (Rescorla & Wagner, 1972). Currently the most influential model of learning, it states that when a contiguous pairing of one or more CS's with a US exists, learning occurs as a result of the discrepancy between what an animal expects the outcome of a given trial to be and what the actual outcome of the trial is. Larger discrepancies will result in increased values of associative strength between a CS and a US, while smaller discrepancies will increase the value of associative strength to a lesser degree. In other words, the more a CS is associated with a US the less additional association the US can induce, resulting in a gradual decrease in the rate of learning over a series of trials. The model mathematically accounts for this type of behavior in the following equation:

$$\Delta V_A = \alpha * \beta (\lambda - V_A)$$

V_A represents the current associative strength between a particular CS, A, and the US; α the salience of the CS; β the learning rate of the US or the US's ability to promote learning to the CS; and λ the asymptote of learning, or the maximum associative value that can be conditioned to the CS. After each trial, the new value of V_A can be calculated by adding the old value of V_A to the change in the associative value of A:

$V_{old} + \Delta V_A = V_{new}$

The value of V_A will increase. However, over trials as the value of Va continues to become stronger, there will be a smaller difference between the maximum possible associative value (λ) and its current associative value (V_A). Therefore, the rate at which the associative value increases actually declines.

The model can also be applied to explain learning to multiple CS's experienced across trials of conditioning, each of which contributes to an overall associative strength to the US. Changes in any component CS are expressed in the following equation:

$\Delta V_i = \alpha * \beta (\lambda - V_{total})$

where V_i represents the associative strength of one component CS, i, and V_{total} represents the aggregate associative strength of all component CS's during that trial. α, β, and λ represent the same measures as explained above. The value of V_{total} can be calculated using the following equation:

$V_{total} = V_A + V_B + ... V_i$

Therefore, increments in the associative strengths of individual component CS's depend on the overall associative strength of all component CS's combined; as V_{total} approaches λ, increments in each V_i decrease.

The model also accounts for inhibitory learning. The same equations are applied to calculate changes in component CS's associative strengths, as well as changes in the aggregate associative strength of all CS's during a trial. Therefore, just as in trials of reinforcement, learning during any given trial of nonreinforcement will depend upon the aggregate associative

strength of all the CS's present. In order for learning to be inhibitory, however, the value of V_{total} must be decremented. This occurs when the associative strength of at least one component CS has a negative value; therefore, V_{total} will be decremented when this negative associative strength is added to the associative strengths of other cues present during the trial (Rescorla & Wagner 1972; Wagner & Rescorla 1972).

Assuming that λ decreases with decreasing US intensity, the value of λ during trials of nonreinforcement is set to 0. Thus, in order to decrement the associative strength of a CS, according to the equations above, the value of $(\lambda - V_{total})$ must be negative. As the value of lambda is 0 for nonreinforced trials, the value of V_{total} must be positive, which implies that a CS cannot attain inhibitory properties, or a negative value for its component V, if it is nonreinforced in isolation. Rather, the CS must be nonreinforced in compound with other cues that make V_{total} positive. This allows the value of $(\lambda - V_{total})$ to be negative, ultimately decrementing the associative value of component CS's and turning them into conditioned inhibitors. As component CS's accrue more decremented values of V, the overall aggregate associative strength value (V_{total}) will decrease as well. (Rescorla & Wagner, 1972; Wagner & Rescorla, 1972). In the negative contingency experiments conducted by Rescorla (1968a) and described above, a series of shocks were presented to animals, but were always discontinued in the presence of a tone CS, producing conditioned inhibition to the CS. Although there was only one explicit CS, the contextual cues experienced in the experimental chamber served as additional stimuli. Therefore, the arrangement of a negative contingency between the tone CS and the US was established by sometimes reinforcing the background CS's, but never presenting reinforcement during compound presentations of the tone CS and background CS's. This

allowed the value of ($\lambda - V_{total}$) to be negative, decrementing the associative value of the component tone CS and turning it into an inhibitor.

The contiguity-based Rescorla-Wagner model can systematically predict experimental outcomes on a trial-by-trial basis, making it one of the most widely accepted theories explaining excitatory and inhibitory conditioning. However, it does not account for the ways in which varying durations of events impact learning (Balsam, Drew & Gallistel, 2010). Alternative models have since been developed to account for the important role of temporal intervals in creating conditioned responses. These models are based on the findings of studies that reveal the capability of animals to learn about time, as well as the various ways by which time affects associative learning.

Studies by Skinner (1938) showed that animals are capable of learning about the temporal information provided by environmental stimuli. He observed a "scalloping phenomenon" in the curves that represented an animal's rate of responding over a fixed interval of time, indicating the animal's ability to learn the temporal information provided by environmental stimuli. On a Fixed-Interval (FI) schedule of reinforcement, an animal receives a reinforcer after the first response following the passage of a specific period of time determined by the experimenter. Unlike an FI schedule, a Variable-Interval (VI) schedule delivers reinforcers after the first response following an average determined length of time. Skinner noticed that animals exposed to an FI schedule increased responding as the end of the fixed interval approached, compared to animals exposed to a VI schedule. This created an upward scallop in the data curve that depicted the cumulative record of responses over time, suggesting that the animals had a knowledge of the duration of the interval, as evidenced by engagement in anticipatory behavior in preparation for the onset of reinforcement.

Other studies display findings that suggest a strong temporal influence on learning. In 1965 Trapold, Carlson and Williams explored the scalloping phenomenon further by examining the conditions that affected the latency to which it occurred. Two groups of rats were given appetitive reinforcers on either a fixed or variable schedule of 2 minutes. When rats were subsequently shifted to an FI-2 schedule of reinforcement for bar-pressing, the groups that had been previously pre-trained with reinforcers delivered on an FI schedule were faster to acquire a scallop in responding than those pre-trained with reinforcers delivered on a VI-schedule, indicating the strong influence of the temporal information conveyed by cues on the ways in which animals exhibit behavior.

Subsequent evidence regarding an animal's ability to learn time, as well as the important role of time in associative learning is seen in a study conducted by Brown, Hemmes & Cabeza de Vaca (1997). Results show that varying durations of trace intervals, or the intervals between the offset of the CS and onset of the US, occurring after the offset of a 6-second CS strongly affect responding, with animals that experienced a short trace responding at higher rates than animals that experienced a long trace. However, both groups of animals, regardless of the duration of the trace period, exhibited knowledge of the temporal information conveyed by their environmental cues, as levels of responding increased throughout the duration of the trace period. Therefore, temporal information affects the animal's rate of responding, in addition to the animal's knowledge regarding the time that elapses between presentations of reinforcers (Brown et al., 1997).

Balsam et al. (2010) proposed the Temporal Information model of learning, which takes timing into account when explaining conditioned behavior in a mathematical manner. In a random process, uncertainty only depends on an animal's knowledge of the rate at which the US

is going to occur, therefore relying on the animal's ability to discriminate different intervals of time. According to the model, information conveyed by the CS can be predicted by calculating the difference between the uncertainty of when the next US will occur in a certain environmental context, and the uncertainty of when the next US will occur during the presence of a specific CS. Unlike the Rescorla-Wagner model, which assumes that the rate of the US during the overall context is equal to rate of the US during a given CS, the Temporal Information model gives the CS the ability to increase the animal's information about the timing of the US. This model, however, accounts mainly for excitatory learning, proposing that excitation is dependent on the delay to reinforcement from the onset of a cue, compared to the overall delay from one reinforcer to the next. Little is known about the ways in which temporal information conveyed by an inhibitory CS can affect conditioned responses. This study examines the issue of inhibitory learning and time, specifically the ways in which inhibitory behavior is affected by CS's of differing durations.

The first experiment will examine responding to four different fixed durations of nonreinforced CS's: of 10 seconds, 20 seconds, 40 seconds and 80 seconds. The second experiment will examine the effect of long and short, fixed and variable nonreinforced CS's on inhibitory behavior.

Experiment 1: CS-Duration

Subjects. Forty male Sprague-Dawley rats were housed in groups of two in a colony room on a 12:12 hour light:dark cycle. The rats were fed in their home cages for one hour after experimental sessions, which occurred five days during the week. On weekends the rats received an unlimited supply of food. Water was available ad lib in the home cages. Rats were

approximately 9 weeks old at the start of the first training session and had been handled for one week prior to testing.

Apparatus. Eight identical experimental chambers (30.5 cm x 24.1 cm x 21.0 cm) located in ventilated and soundproof boxes were used during experimental procedures. Each chamber was equipped with a speaker, a house light, and a magazine food pellet dispenser (Model ENV-203, Med Associates), which delivered pellets into a head-entry-detecting trough (Models ENV-200R7 and ENV-254-CB, Med Associates). A computer running the Med-PC software controlled experimental events and recorded the time during which the events occurred.

Procedure. Rats were tested in five different sets of eight rats each. Rats were assigned randomly into one of the following groups: CS-10, CS-20, CS-40, CS-80, and CSRand. All groups of rats received 2 sessions of magazine training, during which 40 pellets were delivered over the course of 20 minutes, followed by 35 sessions of experimental testing. One session was administered to each rat per day. In the CS-10 experimental group, rats experienced 8 trials of 10-second tones (85dB1000 hz) followed by inter-trial-intervals (ITI) in each session. Forty pellet reinforcers were delivered only during the ITI on a schedule defined by an exponential distribution with a mean of 20 seconds. The length of individual ITI periods was determined by an additional exponential distribution also with a mean of 20 seconds. The CS-20, CS-40 and CS-80 groups were treated identically except for the lengths of each group's respective CS-, with CS-20 rats receiving 20-second tones, CS-40 rats receiving 40-second tones, etc. The fifth group of rats in the remaining CSRand group experienced all four durations of tones in a single experimental session and received an equal rate of reinforcements in the presence and absence of the CS. ITI durations for the Random group were generated by the same schedule as the other four groups. In all groups each session lasted for a total of 8 tone presentations.

Data Collection. The time of occurrence of each head entry and the time of onset and termination of each tone CS and pellet US were recorded.

Experiment 2: Fixed vs. Variable CS-Duration

Subjects. Thirty-two male Sprague Dawley rats were housed, fed and handled under identical conditions as the rats from the previous experiment.

Apparatus. In the first phase of Experiment 2, eight experimental chambers identical to those used in Experiment 1 were used. However, in the second phase of the experiment, one fixed response lever (Model ENV-110M, Med Associates) was installed to the left of the pellet dispenser.

Procedure. Rats were tested in four different sets of eight rats each. Rats were assigned randomly into one of the following groups: CS-30 Fix, CS-30 Var, CS-50 Fix, CS-50 Var. All groups of rats received 2 sessions of magazine training, identical to the training in the previous experiment, followed by 35 sessions of experimental testing. In groups CS-30Fix, CS-50Fix, rats were exposed to tones of fixed durations and in groups CS-30Var and CS-50Var tones were of variable durations. CS durations were fixed at either 30 or 50 seconds, or variable at either 30 or 50 seconds. In the variable groups the duration of the tone was determined by an approximately exponential distribution with the appropriate average. Consequently, the likelihood of the tone ending at any point in time was constant. As in the previous experiment all subjects received a total of 40 pellets on an exponential distribution with a mean of 20 seconds during the ITI and no pellets curing the CS- presentations. After 35 sessions of inhibitory tone exposure, the fixed levers were placed into the experimental chambers, and rats

were trained to bar press for food on a VI-30 schedule of reinforcement, followed by 7 sessions of a CS- training identical to the sessions of the first experimental phase.

Data Collection. The time of occurrence of each head entry and the time of onset and termination of each tone CS and pellet US were recorded.

Results

Experiment 1: CS- Duration

Responding was measured by examining the rate in seconds at which animals poked their heads into the feeder. As a Head Entry (HE) signals the anticipation of a pellet reinforcer, we hypothesized that HE rates during inhibitory CS-'s would be low compared to HE rates during the excitatory ITI periods. To quantify the comparison between CS- and ITI responding, Difference Scores were obtained by subtracting ITI per second HE rates from CS- per second HE rates. As ITI rates (which were hypothesized to be high relative to CS- rates) were subtracted from CS- rates, negative difference scores would indicate inhibition of responding. Furthermore, this would allow for the examination of the pattern of responding throughout seconds of the CS-.

To evaluate the progression of inhibitory responding throughout experimental sessions the largest negative Difference Score for each rat, during each trial of each day was examined. These most negative score across days is plotted in Figure 1. A repeated measures analysis of variance (Day x Group) yielded a significant effect of Day ($F(6)= 16.913$, $p<.05$), a significant effect of Group ($F(4)= 13.9178$, $p<.05$), and a significant Day x Group Interaction ($F(6,4)= 3.198$, $p<.05$), indicating that inhibitory responding increased over days of testing, and was affected by CS- duration, with increasing durations creating stronger inhibitory responding. Furthermore, the significant Day x Group interaction indicates that the degree to which animals

in groups with long CS- durations, compared to those with short CS-durations, inhibited responding increased throughout days of experimental testing.

After computing Difference Scores for each rat as described previously, scores were further divided into deciles, comprised of ten equal portions of each session's CS-. Twenty second CS-'s, for example, were pooled into bins of 2 seconds each, while 80 second CS-'s were pooled into bins of 8 seconds each. This allowed for the difference scores of all rats to be proportionately comparable to one another despite the four different durations of CS-'s. Each rat's deciles of Difference Scores were subsequently averaged into blocks of 5 sessions, resulting in a total of 7 blocks, as there were 35 daily experimental sessions.

The pattern of responding throughout the CS- during the last block of 5 days was examined (Figure 2). A repeated measures analysis of variance (Decile x Group) yielded a significant effect of Decile ($F(9)=11.611$, $p<.05$) with inhibition mostly increasing as the CS- progressed, a significant effect of Group ($F(4)=6.733$, $p<.05$) with groups that experienced longer CS-durations displaying the most inhibition, and a significant Decile x Group interaction ($F(9,4)=22.935$), indicating that the degree to which animals inhibited responding throughout the progression of the CS- depended on CS- duration. In addition, Figure 2 reveals an interesting pattern of responding in rats that experienced the CS-80 tone duration. In particular, rats displayed increased levels of inhibition during the initial quarter to half of the CS-, but subsequently decreased their inhibitory responding in a gradual manner during the remainder of the CS-, creating a "U-shaped" pattern of responding.

Figure 3 displays the pattern of responding throughout the CS- across blocks of all 35 days. Certain groups displayed very large increases in inhibitory responding throughout the CS-

while others displayed inhibition to a lesser degree, or did not display it at all (Significant Block x Decile x Group interaction $F(6, 9, 4)=25.712$, $p<.05$). Furthermore, groups with shorter durations were either retarded or completely unsuccessful in acquiring an inhibitory response throughout the CS- over the progression of experimental sessions. These group disparities seem to be directly related to CS-duration, as groups with shorter CS- durations were extremely slow to acquire a rather weak inhibitory response throughout the CS-, compared to groups with longer CS-durations, who displayed a rapid acquisition of strong inhibition to the CS- during early experimental sessions.

Additionally noteworthy in Figure 3 is the pattern of responding throughout the CS- over days in the CS-40 and CS-80 groups. As seen in Figure 2, by the end of training these groups displayed a U-shaped pattern of responding, with the CS-80 group appearing to acquire this pattern rapidly and to a very strong degree. Figure 4 exhibits an individual CS-80 rat's pattern of responding over days, making the increasing strength of inhibition, as well as the U-shaped pattern of responding visibly evident: during the initial days of testing, inhibiton is weak, but as the days progress not only does inhibition become stronger, but the U-shaped pattern of responding becomes increasingly pronounced after the onset of its initial emergence.

The acquisition of the U-shaped pattern of responding was assessed in Figure 5, by examining responding over blocks of days at various points throughout the CS-. Specifically, figure 5 shows responding during the 1^{st}, 4^{th}, 7^{th} and 10^{th} deciles of the CS- across training days The CS-40 group of rats began to exhibit an upward ramp during the third block of days (Day 11-15), as difference scores during the 7^{th} and 10^{th} deciles became more positive starting at that point. Rats in the CS-80 group began to exhibit the U-shaped pattern even earlier, as difference scores during the 7^{th} and 10^{th} deciles became more positive around the second block of days

(Days 6-10). The figure also reveals that the CS-80 group displayed a more pronounced U-shape pattern than the CS-40 group, as inhibitory responding during the middle to later deciles of the 80 second CS- decreased to a much stronger degree compared to responding during the later deciles of the 40 second CS-.

It appears that longer CS- durations create increased levels of inhibition (as seen in the CS-80 and CS-40 groups), while shorter durations display low levels of inhibition (as seen in the CS-20 and CS-10 groups). It can be argued that rats who experienced shorter durations did not have sufficient time during the CS- to successfully inhibit motor responses. To asses this, we examined the pattern of responding during the first 10 seconds of each group's CS- during select days of experimental testing. Figure 6 shows that the distinct patterns of responding, characteristic to each group, emerged by the 10^{th} day of testing: the CS-80 group displayed the most inhibition, the CS-40 and CS-20 groups displayed lower levels of inhibition, and the CS-10 and CSRand groups displayed little to no inhibition.

Results Experiment 2: Fixed CS- vs. Variable CS-

Difference Scores were calculated, as described in Experiment 1. It is important to note, however, that for groups of rats that experienced variable durations, CS- HE rates consisted of an average of only the first 30 seconds or the first 50 seconds of each trial, as to allow responding patterns of both Fix and Var groups to be easily comparable to one another.

Firstly, the most negative daily Difference Scores across groups were examined (Figure 7) to track the acquisition of inhibition. A repeated measures analysis of variance (Day x Group) yielded a significant effect of Day ($F(6) = 19.136$, $p < 0.05$), and a Day x Group interaction ($F(6,3) = 3.236$, $p < .05$), indicating that inhibitory responding increased throughout the

progression of experimental sessions, and that the degree of inhibition over days was dependent on CS-duration, with CS-50 Fix and CS-50 Var groups displaying more inhibition to the CS- than CS-30 Fix and CS-30 Var groups.

As in experiment 1, difference scores were subsequently divided into deciles and the days of experimental testing were blocked into groups of 5, as was done in the previous experiment. When examining the pattern of responding throughout the CS- during the last 5 days of testing (Figure 8), it was concluded that the level of inhibition throughout deciles of seconds of the CS- differed across groups, with groups experiencing CS- durations of 50 seconds displaying increased inhibition than groups experiencing CS- durations of 30 seconds. A 3-way ANOVA (Decile x Duration x Time) yielded a significant effect of Decile ($F(9) = 8.389$, $p<.05$), and a significant Decile x Duration interaction ($F(9,1) = 4.259$, $p<.05$). In addition, the pattern of responding throughout the CS- in the CS-50 Fix and the CS-50 Var groups depended on whether the cue was fixed or variable, as an analysis of variance examining those two groups only yielded a significant Decile x Group interaction ($F=5.215$, $p<.05$). As made clear in Figure 8, a U-shaped pattern of responding towards the end of the CS- was seen in the CS-50 Fix group, but not in the CS-50 Var groups. However, there were no significant differences in responding patterns throughout the CS- between the CS-30 Fix and the CS-30 Var groups.

Across blocks of days (Figure 9), groups with longer CS-durations displayed more inhibitory responding than groups with shorter durations, as an analysis of variance yielded a significant Block x Group interaction ($F(6,3) = 2.188$, $p<.05$). Figure 9 shows that patterns of responding throughout the CS- differed among groups as well, with the CS-50 Fix group being the only one of the four groups to acquire over days a pattern of decreasing in inhibition towards the end of the CS-, producing an upward ramp in responding.

Responding at various points throughout the CS- throughout days of experimental testing was examined in the CS-50 Fix and CS-50 Var groups (Figure 10). As in the previous experiment, responding during the 1st, 4th, 7th and 10th deciles of the CS- were considered. Results did not indicate any U-shape pattern in the CS-50 Var group, as responding during the later deciles did not become more positive at any point throughout the experimental sessions, but instead became stronger with the progression of days. Contrarily, rats in the CS-50 Fix group did display a U-shaped pattern, which first began to make an appearance around the second block of days (Days 6-10), as responding during the 7th and 10th deciles decreased throughout the remainder of the experiment.

Discussion

Results strongly suggest that the temporal information provided by inhibitory cues plays an important role in the ways by which animals acquire and exhibit behaviors. Firstly, the strength of inhibitory responding is affected by temporal information, as results showed that large CS- durations produced the most inhibition and short CS- durations produced the least. Secondly, the speed at which the inhibitory response is acquired is affected by temporal information, as longer CS- durations produced more rapid acquisition of inhibitory behavior than shorter CS- durations, displaying very negative Difference Scores during the first few days of experimental testing.

Lastly, the pattern of responding throughout the CS- is affected by temporal information, as a strong "scalloping" pattern emerged throughout the CS- in groups of rats who experienced long, fixed CS-durations. This indicates that when animals are given sufficient information that allows them to time the duration of the CS- (such as experiencing a fixed rather than variable

cue), the CS- can serve as both an inhibitor and an excitor. Initially, the CS- produces inhibition, as evidenced by the negative Difference Scores during the initial seconds of the CS-. (As mentioned previously, the strength of this inhibition was the largest in groups of rats who experienced the longest CS- durations.) At approximately one third to one half of the way into the CS-, however, Difference Scores became more positive, indicating that the animals were anticipating the onset of the ITI, the period during which reinforcers were delivered. This pattern of strong initial inhibition followed by gradual decreases in inhibition is manifested in the form of a scalloped curve when Difference Scores are plotted across seconds into the CS-. In addition, the speed at which the upward ramp in responding was acquired – an indicator of excitatory behavior, as animals exhibited increases in food anticipatory responding – was more rapid for groups of animals with long CS- durations. Animals who received variable CS-'s did not have adequate information to accurately learn the durations of the CS-. Therefore, they did not exhibit an upward scallop in responding, but rather maintained steadily increasing levels of inhibition throughout the duration of the CS-, indicating that this phenomenon is, in fact, mediated by temporal information.

According to results, CS- duration influences the strength of inhibition over days, the speed at which the inhibitory response is acquired, the pattern of responding throughout the CS-, and the acquisition of said CS- patterns. The longer the CS- duration, the stronger the strength and pattern of responding, and the more rapid the acquisition of responding. Results provide strong evidence that it is, in fact, the information provided by the CS-duration driving these behaviors, rather than other confounding variables such as deficits in motor responses. It could be argued that groups of animals with shorter CS- durations exhibited little to no responding due to the failure to inhibit their motor responses during a miniscule 10-second CS- interval. As

animals were receiving reinforcement during the ITI, head entry responses during this period remained high; when the ITI was terminated for an abrupt 10 or 20 seconds, compared to a lengthy 40, 50 or 80 seconds, animals may not have had sufficient time to inhibit the motor response of food-anticipatory behavior. However, after examining responding in all groups of the first experiment during the first 10 seconds of the CS- over days it becomes clear that low levels of inhibition were not due to a failure to inhibit responding during the early seconds of the CS-; distinct group patterns of responding during the first 10 seconds emerged by the 10^{th} day of testing.

Although results present strong evidence that varying the CS- duration can influence inhibition, there do exist minor flaws that must be addressed prior to asserting definitively our conclusions. Firstly, we assumed that the exhibition of higher rates of trough head entries during the ITI compared to those during the CS- indicated that the CS-'s were inhibitors of reinforcement. However, previous research suggests that when measuring the degree to which a stimulus is an inhibitor, factors such as attention should be considered. When the US is presented in the absence of the CS- (as in an inhibitory paradigm), the animal may attend to a variety of contextual stimuli other than the CS-, possibly causing the suppression of responding during the CS- as a result of the animal's failure to attend to the uninteresting and non-rewarding CS- (Rescorla, 1968b). To address this confound Rescorla proposed two techniques for measuring inhibitory behavior in a more accurate manner. They include the Summation and Retardation Tests (1968b). In the Retardation Test, an animal is presented with an inhibitory cue for several trials. The same cue is subsequently turned into an excitor by repeatedly presenting it in compound with reinforcement. Measuring the latency of the animal to display excitatory behavior provides information regarding whether or not the cue previously contained inhibitory

qualities. If the cue was, in fact, inhibitory, the animal would exhibit retarded acquisition of responding to the cue, compared to animals that did not have inhibitory training on previous trials. The Summation Test involves presenting animals with trials of an inhibitory CS, followed by trials of an additional excitatory CS. If the CS is an inhibitor, the two stimuli compounded together should allow for the inhibitory CS to reduce the probability of the excitatory CS in producing its usual CR. The Retardation and Summation tests, taken together, eliminate confounds of the effects of a novel compound stimulus, and of failure to attend to the CS- (Rescorla, 1968b).

To further test for inhibition in our study, Retardation and Summation Tests could have been performed following the 35 sessions of inhibition testing. A Retardation Test could have consisted of continuing tone CS presentations, but delivering pellets only during the CS, as opposed to the ITI. If animals display retarded acquisition of excitatory learning to the tone CS's, we would more confidently state that the CS-'s during experimental testing served as conditioned inhibitors. A Summation test could have been performed by presenting the animals with an additional CS, such as a lever, during the days following experimental training. Once the animals learn that pressing the lever leads to reinforcement, the new lever CS will accrue an excitatory value. When the lever and the original tone CS- are presented together, we would be able to conclude that the tone CS- is an inhibitor, if the excitatory response to the lever is suppressed.

A second limitation to this experiment includes the lack of control for total CS- exposure across groups. Previous research by Katzev and Berman (1974) has suggested that CS exposure influences conditioned behaviors in rats. In particular, when rats received extensive exposure to a CS (an auditory tone that signaled the termination of shock when a target "avoidance" response

was exhibited), the extinction of such avoidance responses in a subsequent extinction phase (a period during which no shock was presented, regardless of whether target avoidance responses were made or not) was very rapid. However, when rats did not receive extensive CS exposure, subsequent extinction of the conditioned avoidance response occurred more slowly. This importantly indicates that the amount of overall time that an animal is exposed to a CS can affect the way behaviors are learned. In this study, animals in the CS-80 group received eight times the amount of total tone exposure, compared to those in the CS-10 group, possibly giving the animals who experienced the longer duration more opportunity to learn about the CS-'s temporal information. Future studies examining the effect of varying CS- durations on inhibition should control for this by allowing groups with short CS- durations to receive equal amounts of exposure to the CS- by increasing the number of trials per session.

To recapitulate, the data presented in this study indicate that varying the duration of a CS- affects inhibitory learning, in particular, the strength and pattern of responding, as well as the speed at which such behaviors are acquired. This importantly suggests that animals use temporal information, rather than the information provided by the contingency of the relationships between stimuli, to guide learning. As mentioned above, Rescorla proposed that inhibitory learning occurs when there is a negative contingency between a CS- and US, or when there is a higher probability of US occurrence during the CS-'s absence, relative to periods during the CS-'s presence, and that stronger negative contingencies will result in greater conditioned inhibition (Rescorla, 1968a; Rescorla 1968b). In the study presented in this paper, contingencies between the CS- and US remained constant across all groups. According to Rescorla's Contingency Theory, all groups should have displayed equal amounts of inhibition to their respective CS-'s. Such results were not obtained, which provides evidence that factors other than contingency

serve to underlie the process of inhibition. In addition, the mathematically precise Rescorla-Wagner Model would not be able to account for the results that we obtained in this study, as the model does not consider times. The model would treat all CS-'s equally; nonreinforced tone stimuli presented in compound with reinforced background stimuli would cause the tone to become an inhibitor. Assuming that values for alpha and beta are held constant, animals would acquire negative associative strengths at the same rates (Rescorla & Wagner 1972; Wagner & Rescorla 1972). As our results did not demonstrate the acquisition of inhibition at equal rates across groups, this suggests that the Rescorla Wagner Model is lacking in that it does not explain how the temporal information provided by the cue drives the value of its associative strength.

In conclusion, this study provides convincing evidence that temporal information guides inhibitory learning in a variety of ways: it controls the strength of responding, the speed of responding, and the pattern of responding. Furthermore, when enough information is provided to allow the animal to accurately time the duration of stimuli, temporal information can even transform the nature of a cue, turning an initially inhibitory cue into both an inhibitor and an excitor.

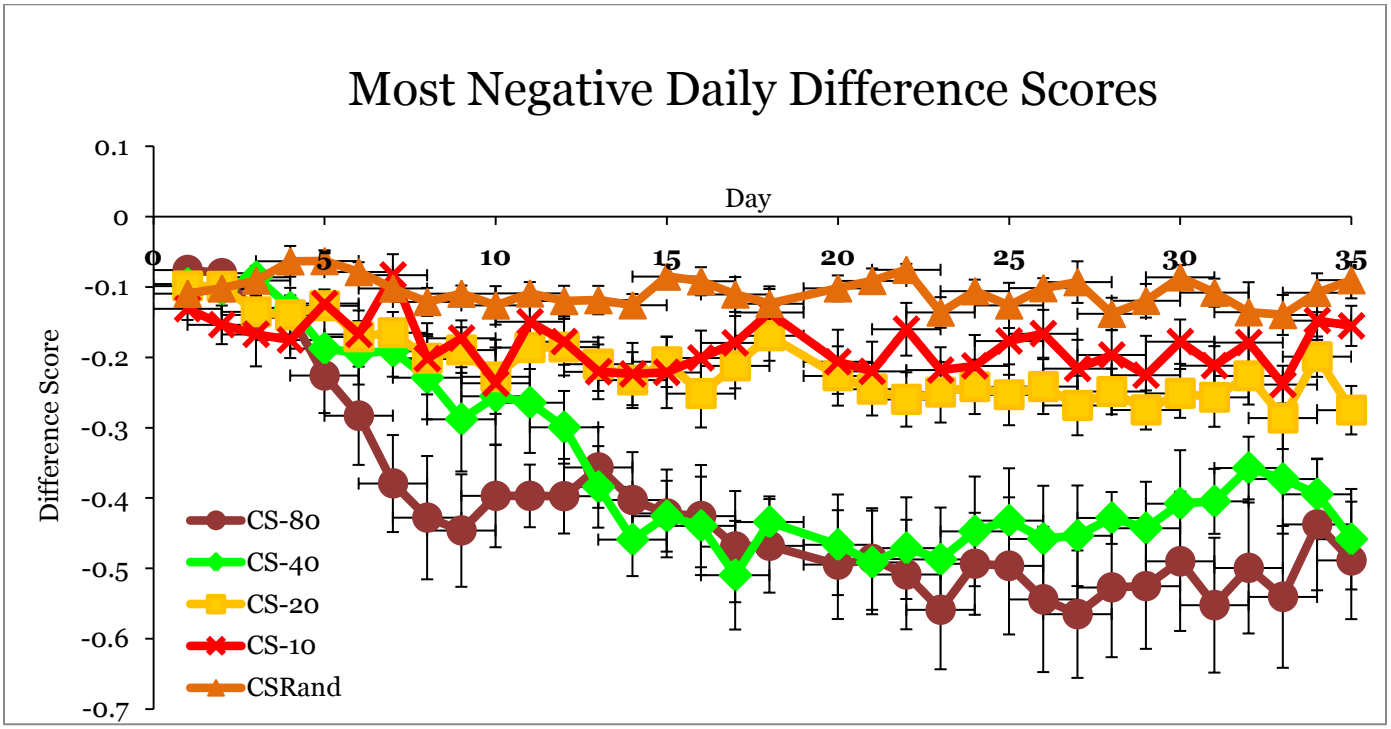


Figure 1: Most Negative Daily Difference Scores. As CS- duration increases, the strength of inhibition throughout days of the experiment increases.

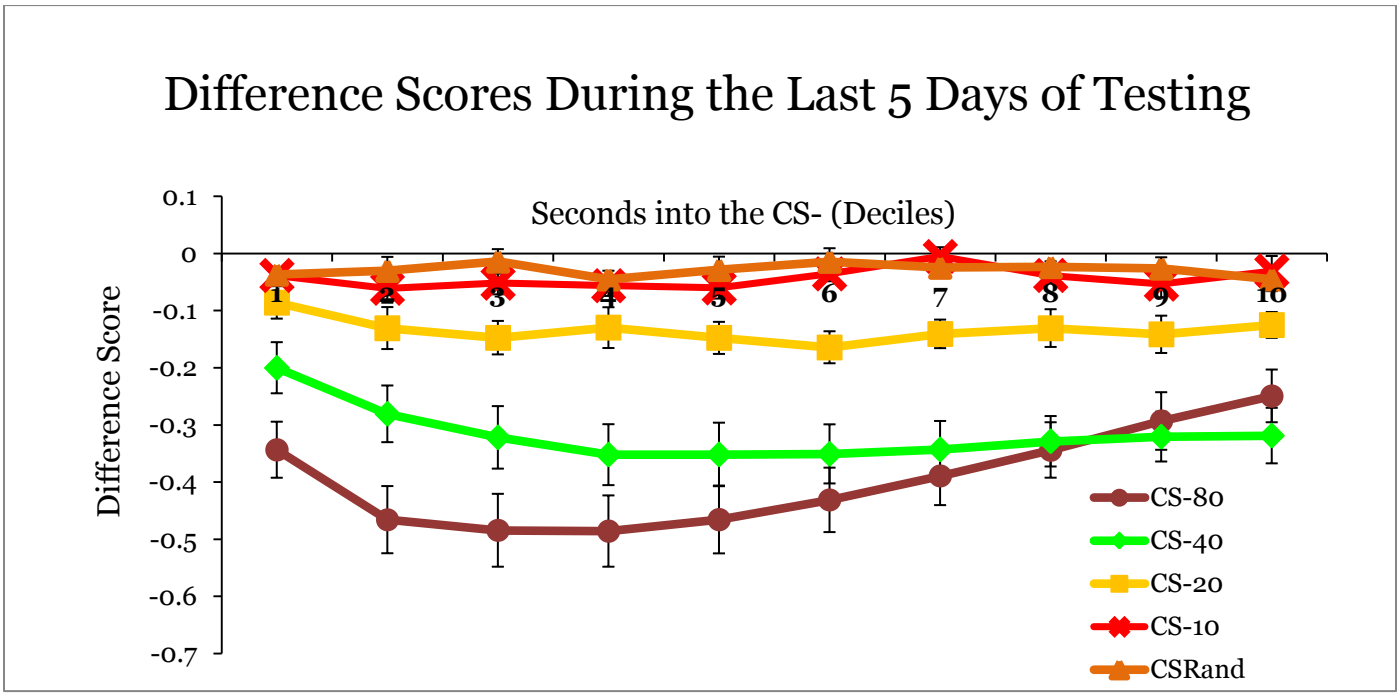


Figure 2: Pattern of Responding Throughout the CS- During the Last 5 Days of Testing. The degree of inhibitory responding across groups differs at various points throughout the CS-. Overall, the groups with the longest CS- durations display the most inhibition. Additionally, the CS-80 group displays a U-shaped pattern of responding.

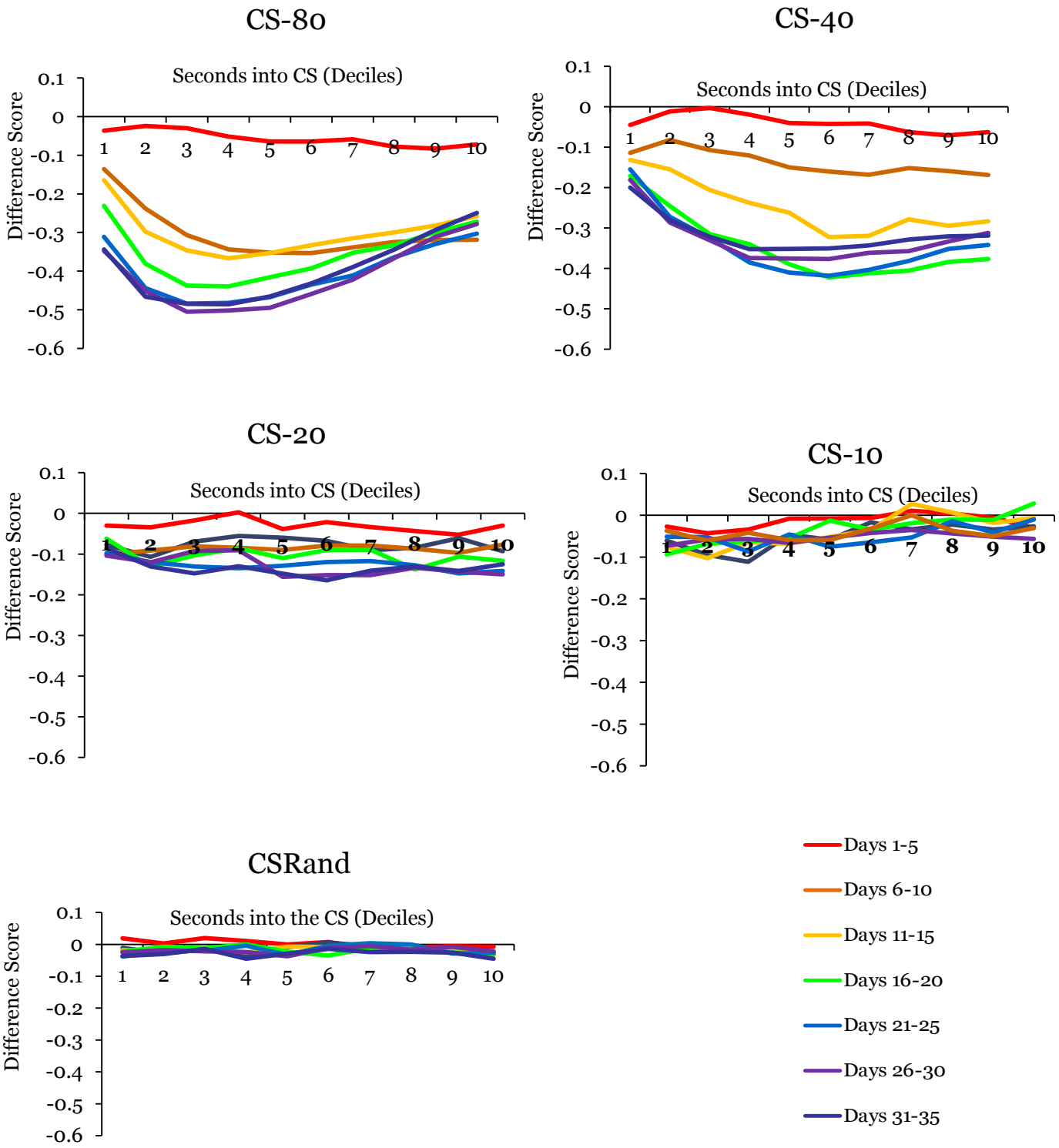


Figure 3: Responding Throughout the CS- over Days. Across blocks of days, certain groups display very strong inhibitory responding while others display inhibition to a weaker degree, or do not display it at all. These group disparities seem to be directly related to CS-duration.

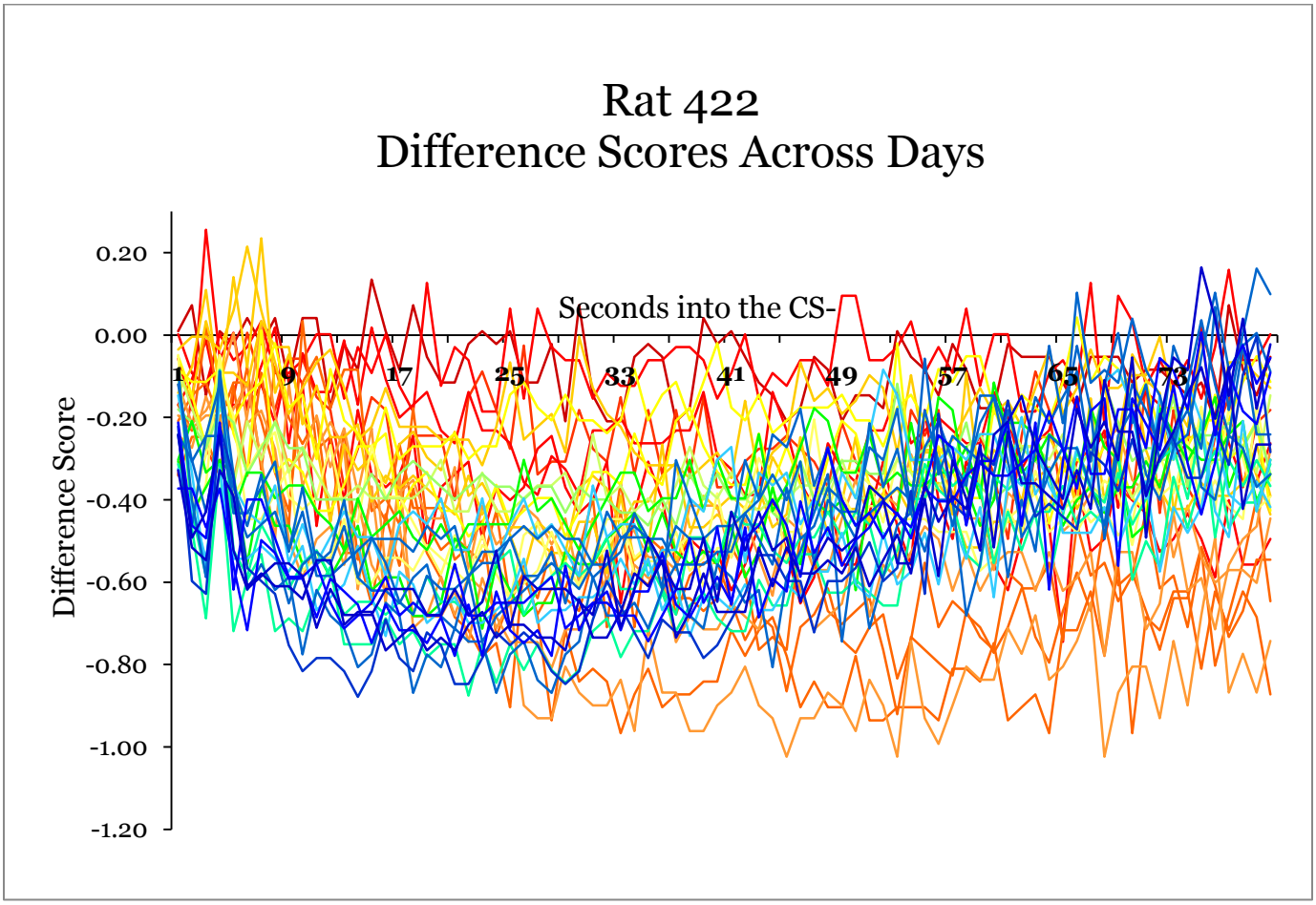


Figure 4: CS-80 Rat, Responding Throughout the CS- Over Days. Each day's responses are represented by a color of the rainbow spectrum; responses marked in shades of red and orange occurred during initial days of the experiment, while responses marked in shades of blue and violet occurred during the final days of the experiment. This rat displays a U-shaped pattern of responding throughout seconds of the CS-, and seems to have acquired such a response about midway through the days of experimental testing.

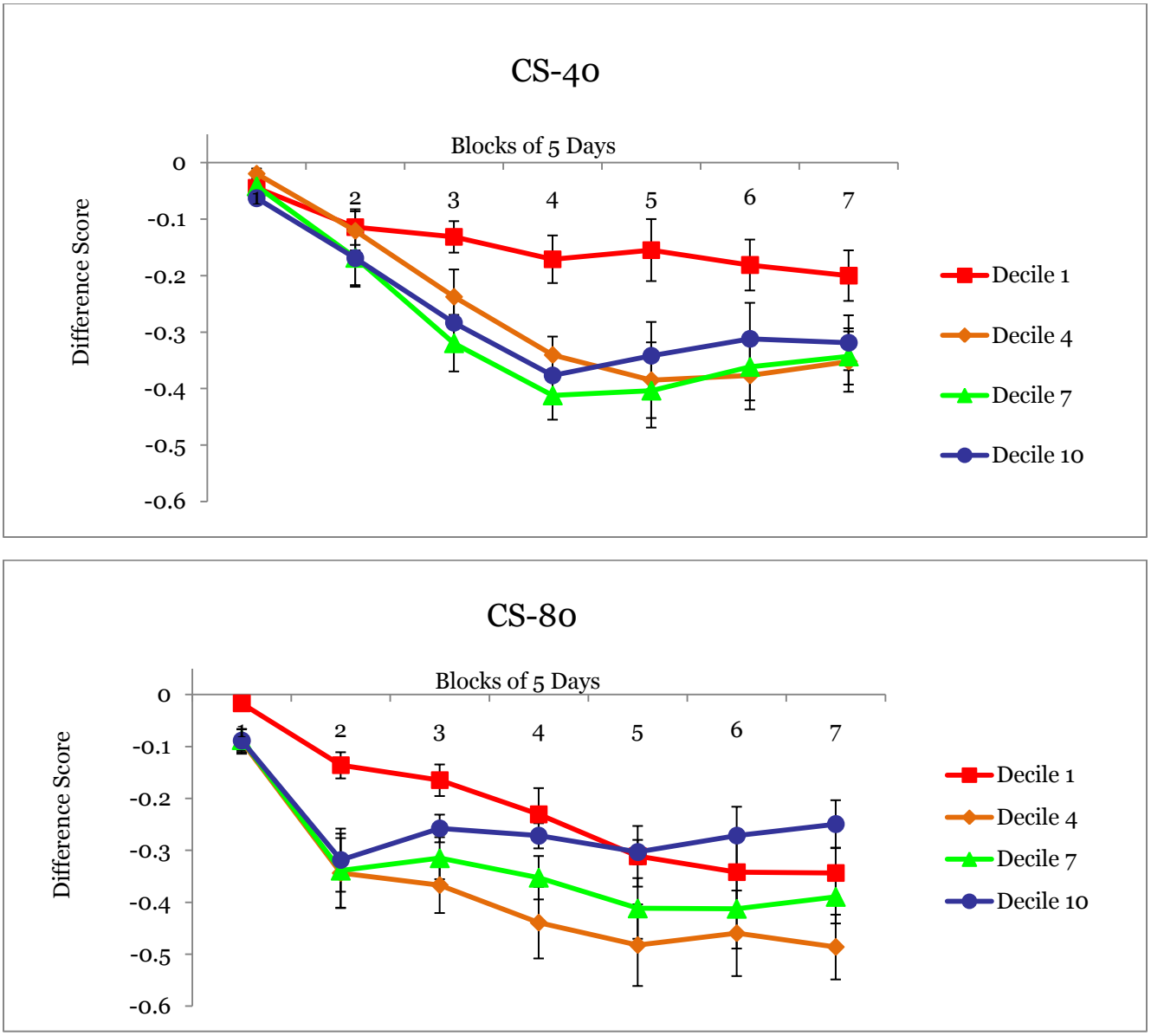
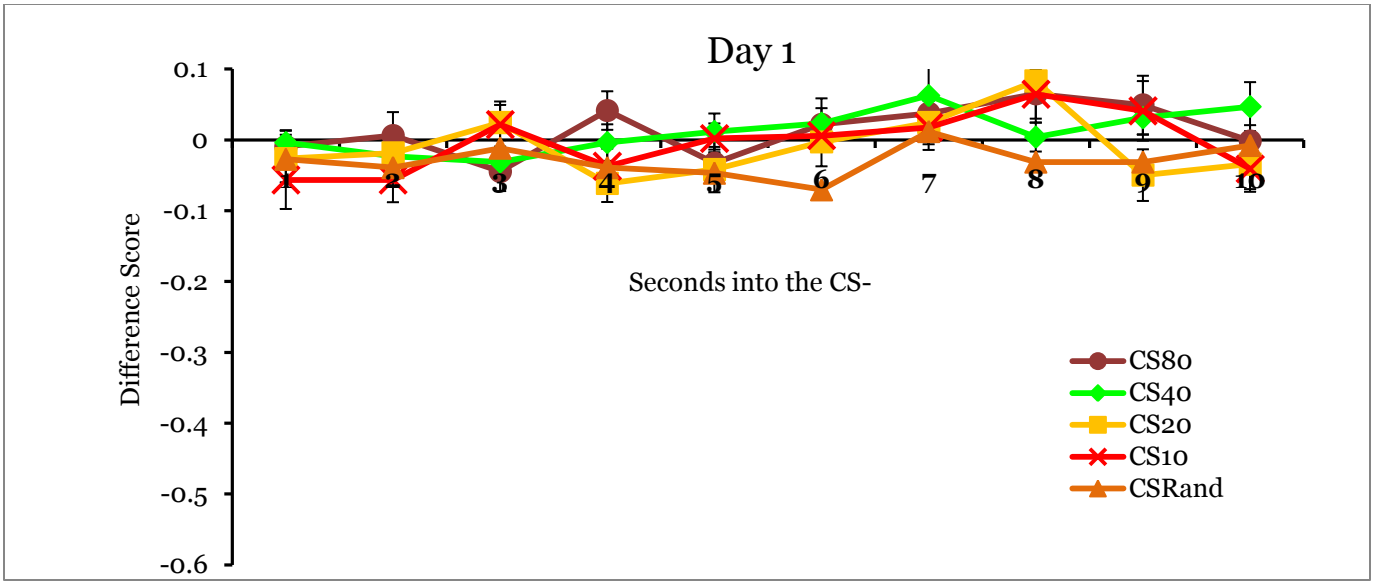
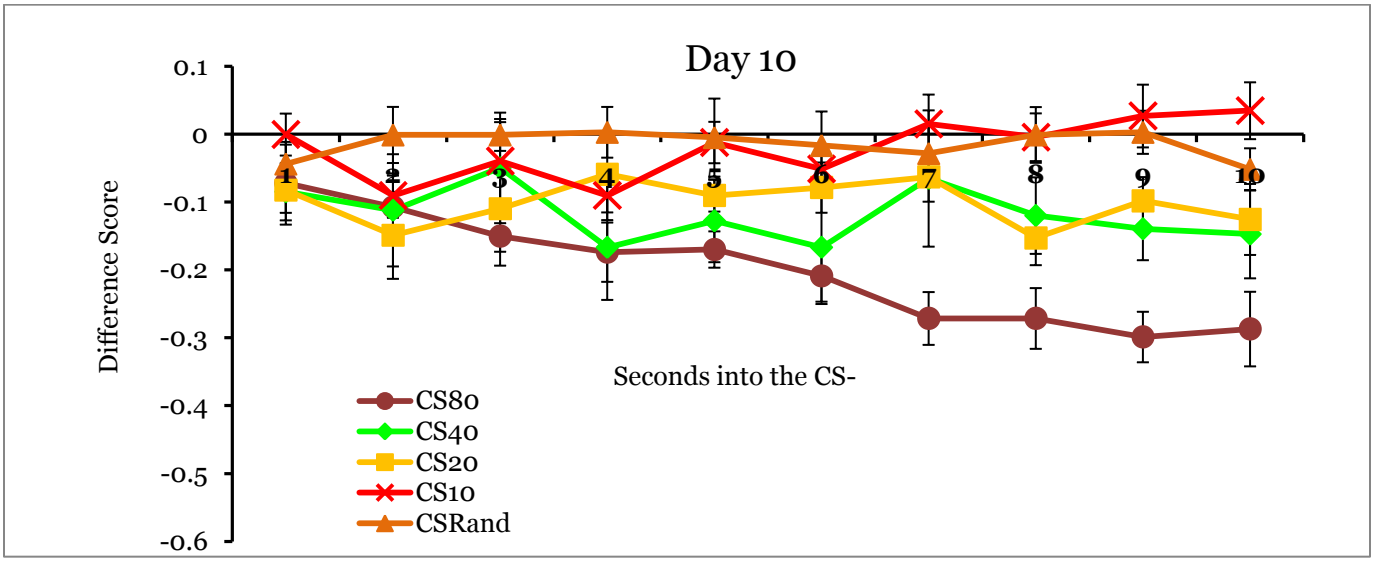
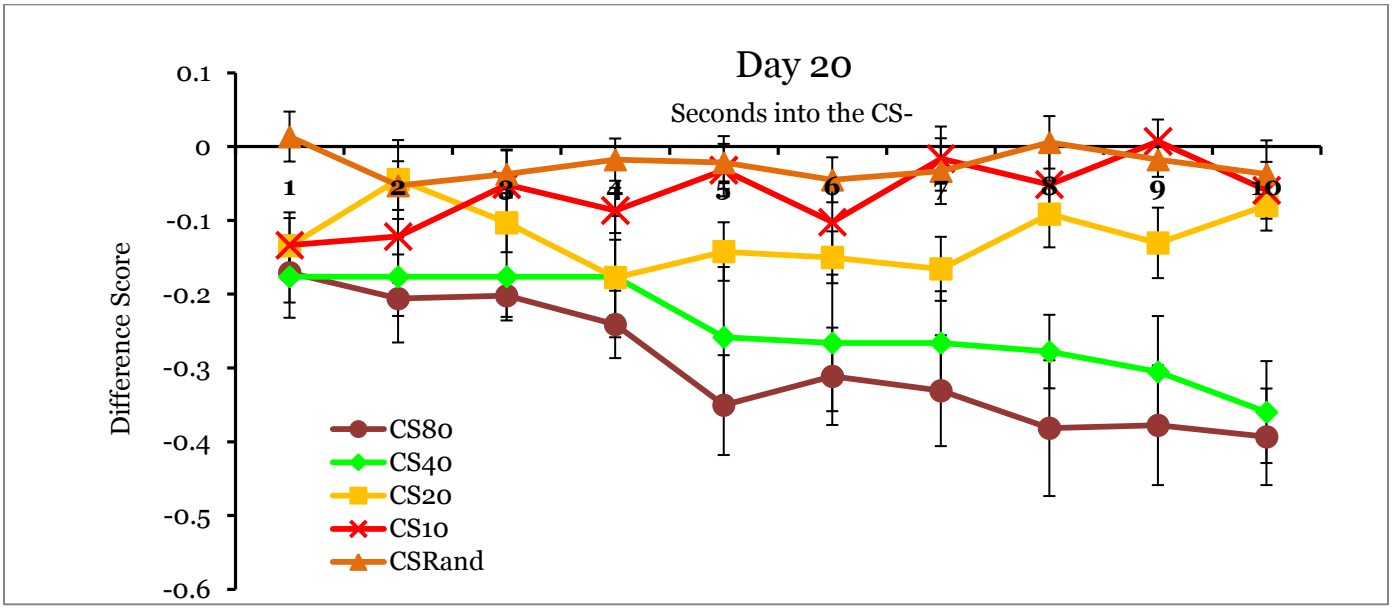


Figure 5: The Acquisition of the Upward Ramp in CS-80 and CS-40 groups. Both groups displayed an upward ramp, as responding during the later deciles of the CS- became more positive across days of experimental testing.


Day 1
Difference Score
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
1
2
3
4
5
6
7
8
9
10
Seconds into the CS-
CS80
CS40
CS20
CS10
CSRand


Day 10
Difference Score
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
1
2
3
4
5
6
7
8
9
10
Seconds into the CS-
CS80
CS40
CS20
CS10
CSRand


Day 20
Seconds into the CS-
Difference Score
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
1
2
3
4
5
6
7
8
9
10
CS80
CS40
CS20
CS10
CSRand

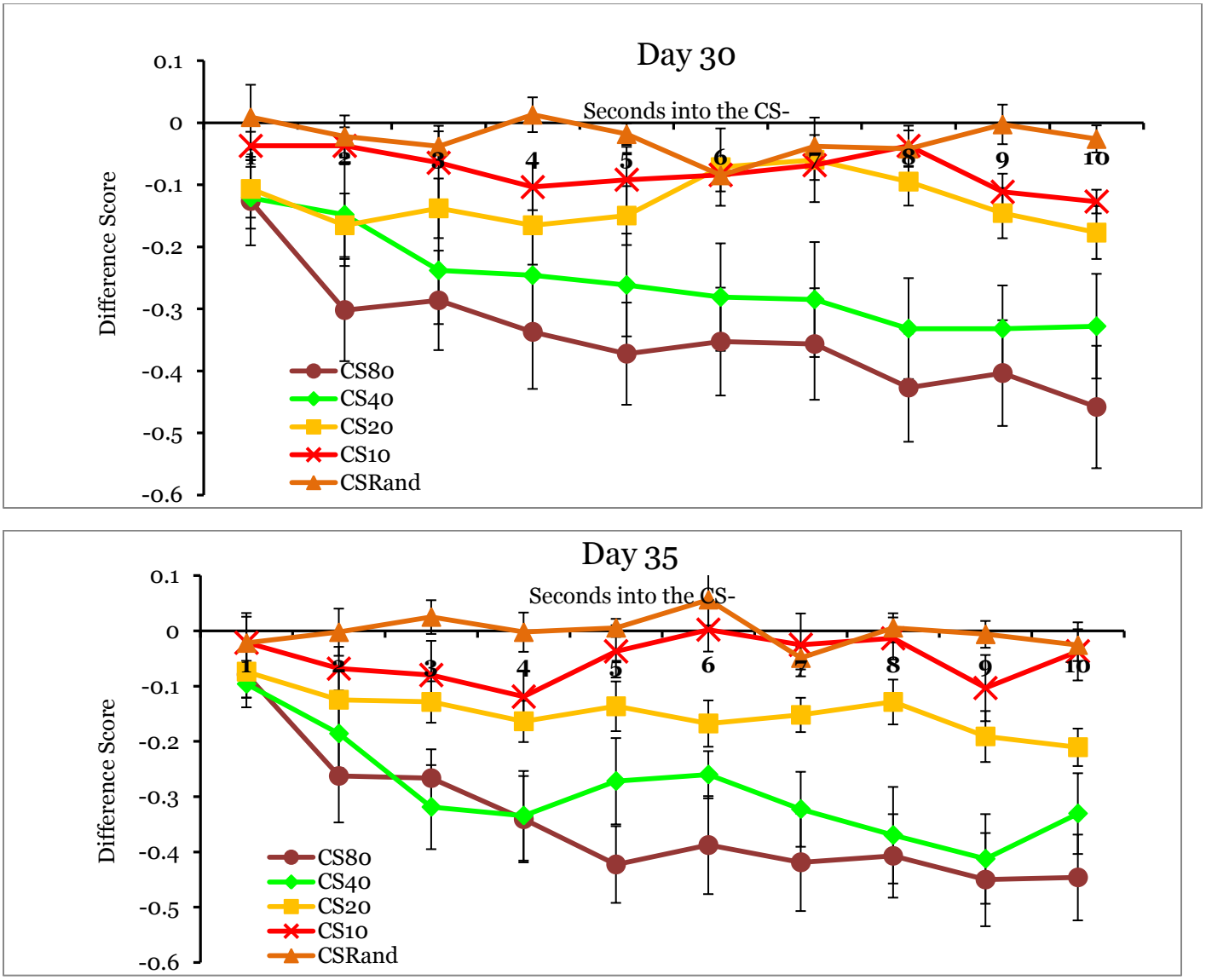
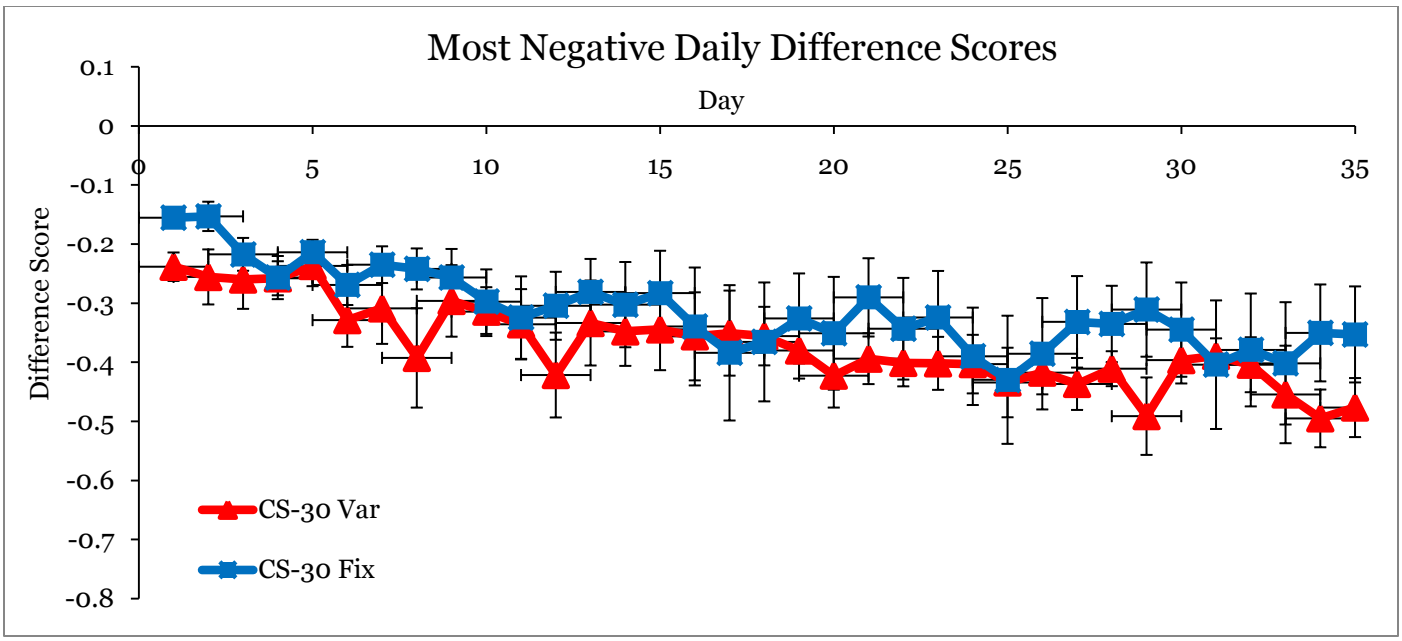


Figure 6: Patterns of Responding During the First 10 seconds of the CS- (Day 1, Day 10, Day 20, Day 30, Day 35). By the 10th day of testing, animals with long CS- durations began to display more negative Difference Scores during the initial 10 seconds of the CS-, unlike animals with short CS-durations.



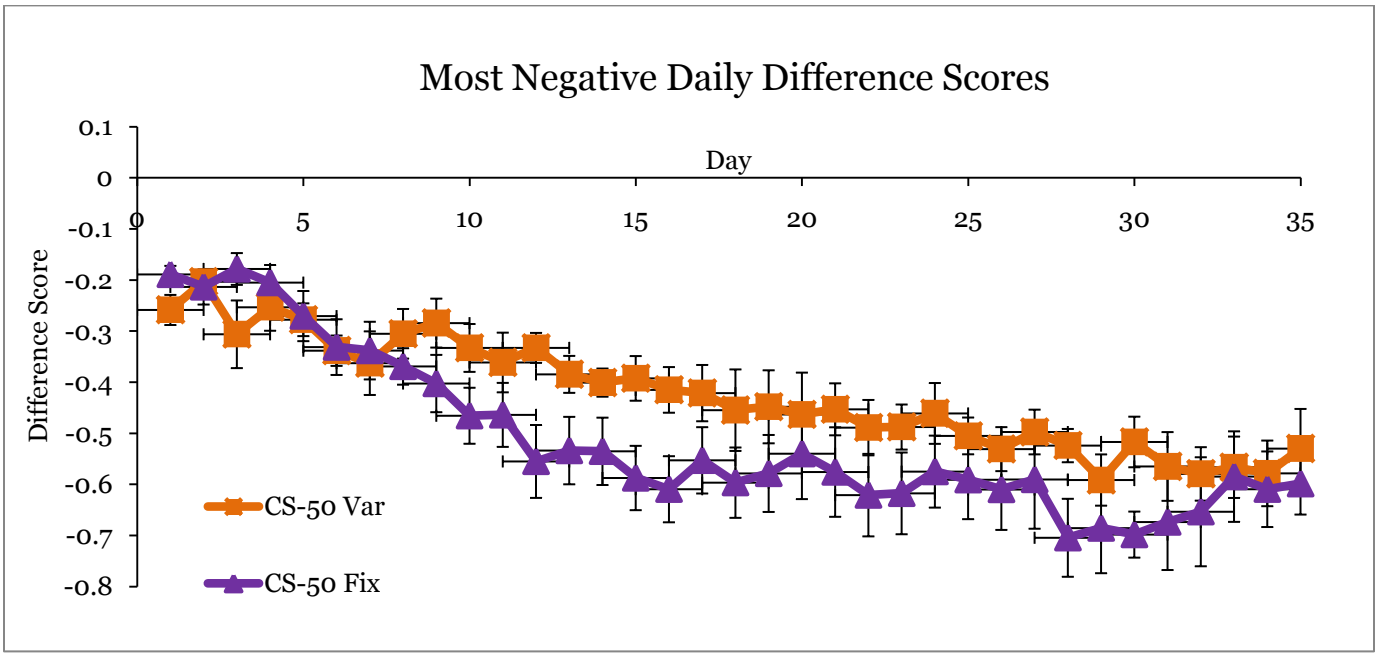


Figure 7: Lowest Daily Difference Scores. As seen previously in Expteriment 1, inhibitory responding increased across days with increasing CS- durations.

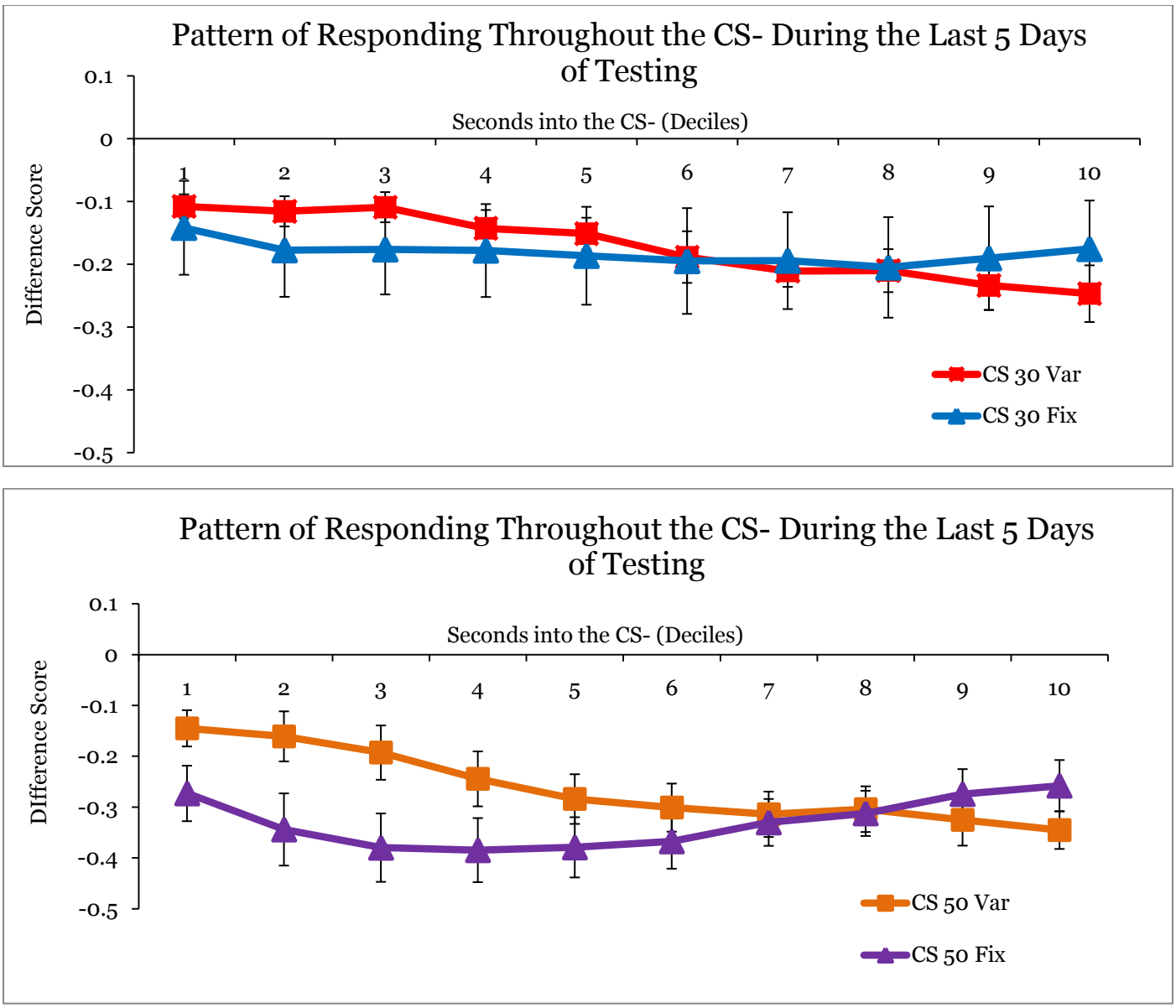


Figure 8: Pattern of Responding Throughout the CS- During the Last 5 Days of Testing. The level of inhibition throughout deciles of seconds of the CS- differed across groups, with long CS- duration groups experiencing greater inhibition than those with short CS- durations. Furthermore, in the CS-50 Fix and CS-50 Var groups, the pattern of responding throughout the CS- depended on whether the cue was fixed or variable.

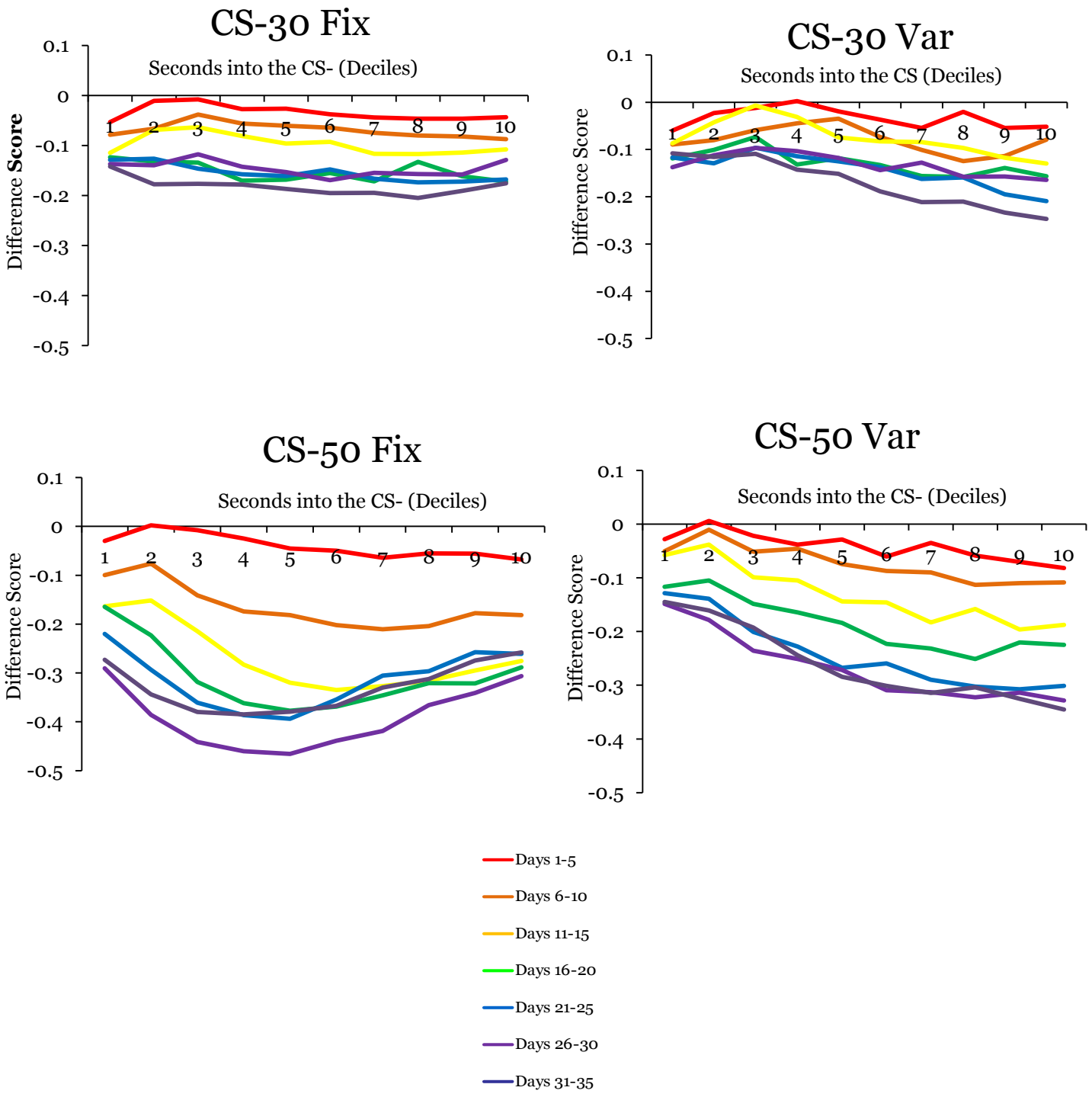
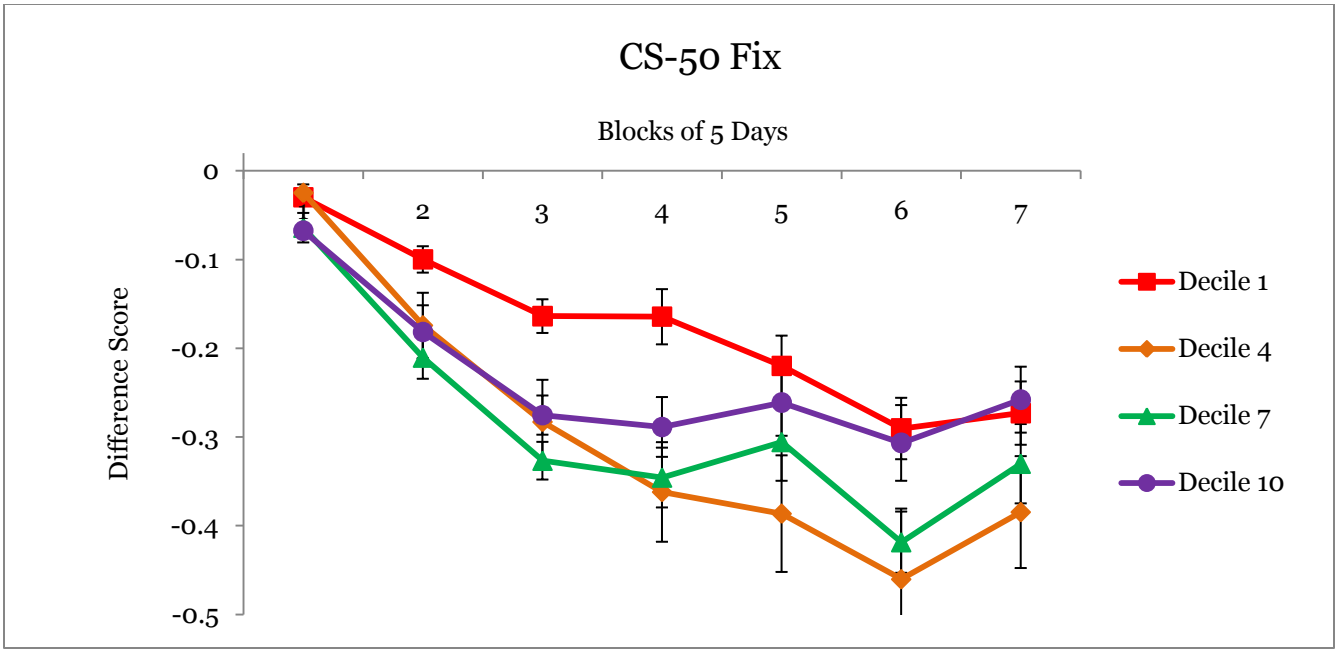


Figure 9: Responding Throughout the CS- over Days. Across blocks of days, groups with longer CS- durations displayed more inhibitory responding than groups with shorter durations. Furthermore, throughout deciles of seconds of the CS-, the patterns of inhibitory responding depended on whether the cue was fixed or variable



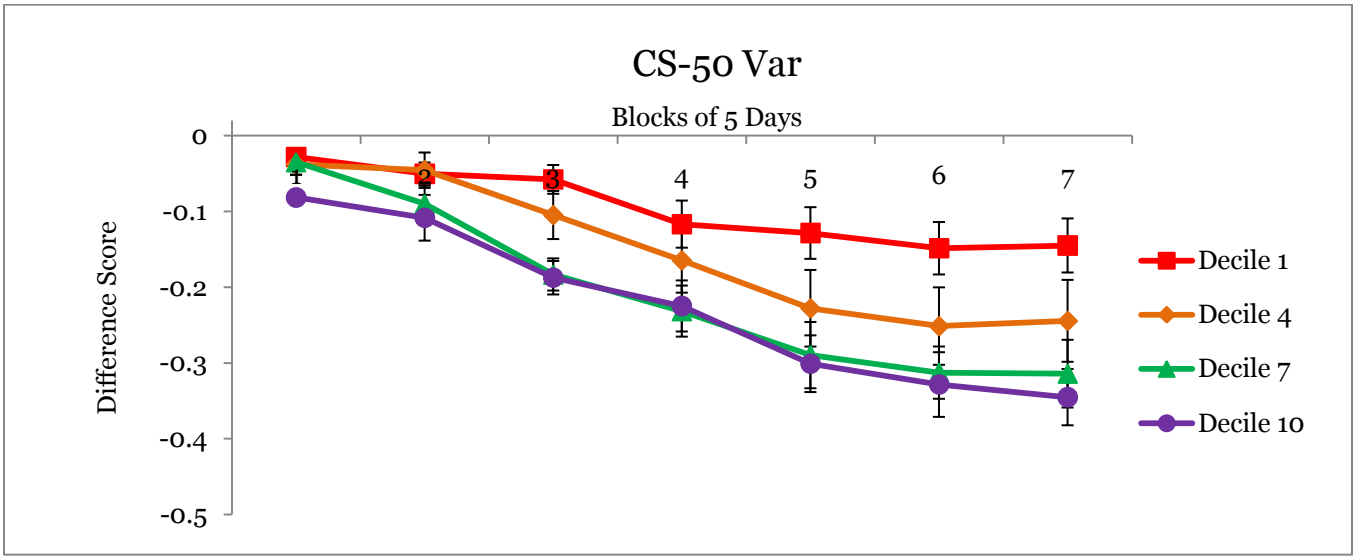


Figure 10: The Acquisition of the Upward Ramp in CS-50 Fix and CS-50 Var groups. Only the CS-50 Fix group displayed an upward ramp, as responding during the later deciles of the CS- became more positive across days of experimental testing.

References

Balsam, P. D., Drew, M. R., & Gallistel, C. R. (2010). Time and associative learning. *Comparative Cognition & Behavior Reviews*, 5, 1-22.

Brown, B. L, Hemmes, N. S., & Cabeza de Vaca, S. (1997). Timing of the CS-US interval by pigeons in trace and delay autoshaping. *The Quarterly Journal of Experimental Psychology*, 50B, 40-53.

Katzev, R. D., & Berman, J. S. (1974). Effect of exposure to conditioned stimulus and control of its termination in the extinction of avoidance behavior. *Journal of Comparative and Physiological Psychology*, 87. 347-353.

Pavlov, I. P. (1927). *Conditioned reflexes* (tr. G. V. Anrep). London: Oxford University Press.

Rescorla, R. A. (1968a). Conditioned inhibition of fear resulting from negative CS-US contingencies. *Journal of Comparative and Physiological Psychology*, 67. 504-509.

Rescorla, R. A. (1968b). Probability of shock in the presence and absence of CS in fear conditioning. *Journal of Comparative and Physiological Psychology*, 66, 1-5.

Rescorla, R. A., & Wagner, A. R. (1972). A theory of Pavlovian conditioning: *Variations in the effectiveness of reinforcement and nonreinforcement*. In A. H. Black & W. F. Prokasy (Eds.), Classical Conditioning II: Current Research and Theory (pp. 64-99). New York: Appleton-Century-Crofts.

Skinner, B. F. (1938). *The behavior of organisms*. New York: Appleton-Century-Crofts.

Trapold, M. A., Carlson, J. C., Myers, W. A. (1965). The effect of noncontingent fixed and variable reinforcement upon subsequent acquisition of the fixed interval scallop. *Psychonomic Science*, 2, 261-262.

Wagner, A. R. & Rescorla, R. A. (1972). *Inhibition in Pavlovian conditioning: application of a theory*. In R. A Boakes & M. S. Halliday (Eds.), Inhibition and learning (pp. 301-336). London: Acedemic Press.